

Wilting and inoculation of *Lactobacillus buchneri* on intercropped triticale-fava silage: effects on nutritive, fermentative and aerobic stability characteristics

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This study investigated the effects of wilting and *Lactobacillus buchneri* inoculation on fermentation end products, DM recovery, nutritive characteristics and aerobic stability in organically grown triticale-fava bean intercrop silages. For this purpose, a bi-crop of triticale (*× Triticosecale Wittm.*) and fava bean (*Vicia faba* L.) was established on an old low-input mixed sward (*Lolium perenne-Trifolium repens*). The association of triticale and fava bean in winter crops and wilting forages before ensiling improved ensilability characteristics. Wilting for 24 hours before ensiling avoided effluent losses during the fermentation process and reduced ammonia nitrogen production. Inoculation with *Lactobacillus buchneri* 40788, for a final application rate of 1×10^5 cfu g⁻¹ of fresh forage ensiled in laboratory silos during 80 days, promoted a higher CP concentration. Furthermore, it promoted changes in the concentration of fermentation end products, decreasing lactic acid and increasing acetic and propionic acids. The effects of *Lactobacillus buchneri* on aerobic stability were not confirmed in this study. Wilting depressed, but did not inhibit the activity of *Lactobacillus buchneri* in the fermentation process.

Key-words: *Lactobacillus buchneri*, wilting, ensilability characteristics, effluent production, nutritive value, organic management

Introduction

In humid temperate areas with oceanic climatic conditions, the most common rotation crops have traditionally been Italian ryegrass-maize (*Lolium multiflorum* Lam.-*Zea mays* L.) due to their high productivity. However, the negative effect on soil fertility of this rotation has prompted the introduction of new forage crops that provide an alternative to Italian ryegrass as a winter crop. Some alternatives have been mixtures of grasses with legumes which are able to fix nitrogen in the soil, thereby reducing nitrogen requirements and associated with environmental benefits (Vanotti et al. 1997). Moreover, farmers have become increasingly interested in cereal-legume intercrops for winter-feeding in order to improve the efficiency of production systems. In this respect, Adesogan et al. (2002) have shown pea-wheat bi-crop silages to be both high yielding and environmentally benign forages that promote higher dry matter intake (DMI) and greater nitrogen contents than grass silages. Our research group has tested several legumes in association with winter cereals, including pea-wheat bi-crops, obtaining the best results when combining triticale-fava bean (x *Triticosecale* Wittm.-*Vicia faba* L.) in rotation with maize (Pedrol and Martínez 2003, Martínez-Fernández et al. 2008). In this intercrop, the cereal contributes dry matter and water soluble carbohydrates that improve ensilability characteristics, thus reducing effluent production and minimizing environmental risks. However, this intercrop yields a lower nutritive value than a legume crop alone.

As regards the triticale-fava bean intercrop, the optimal time for mowing is at the initial grain stage for triticale and immature legumes for fava bean (Argentería et al. 2004). In these phenological stages, high energy and protein values are available as well as lower contents in DM (Arzani et al. 2004, Lloveras-Vilamanya 1987). For this reason, pre-wilting the intercrop before ensiling seems to be necessary. In fact, the higher the DM of the crop, the lower the bacterial activity and the role of fermentation acids in preservation (Wolford 1984).

Some biological additives, such as homofermentative bacteria, improve fermentative activity

during the fermentation process, silage nutritive value and animal response in terms of milk and meat production. However, these additives can reduce silage stability during storage and after opening. These effects are due to the lactic acid produced throughout the fermentation process, which is metabolized by some species of yeast and mould upon exposure to oxygen (Combs and Hoffman 2001), and reduced production of antifungal factors (Kung et al. 2003). When air infiltrates the silage during storage, the growth of aerobic micro-organisms is stimulated and the process of aerobic deterioration is initiated, leading to dry matter losses of feed, decreasing the feed nutritive value and probably also reducing voluntary feed intake. Yeast acid-tolerant and occasionally acetic acid-tolerant bacteria are the main micro-organisms responsible for consuming nutrients and fermentative residual products, increasing temperature in the silage mass and reducing dry matter and energy (Taylor et al. 2002, Reis et al. 2005).

In contrast, some commercial additives produced with heterofermentative *Lactobacillus* cultures have demonstrated an ability to inhibit fungal growth (Nishino and Hattori 2005) and have been used to improve the aerobic stability of silages after long-term storage (Kung and Ranjit 2001). Their activity is related to the presence of acetic acid, which inhibits the growth of specific species of yeast responsible for heating upon exposure to oxygen and also decreasing losses during the fermentation process and improving animal production (Kung et al. 2003). Different studies have shown the efficiency of *Lactobacillus buchneri* 40788, which, when added to fodder that has been harvested, increases acetic fermentation and reduces fungal contamination, thereby improving the aerobic stability of silage by reducing yeast growth (Combs and Hoffman 2001, Kung and Ranjit 2001, Kleinschmit and Kung 2006). The positive impact of *L. buchneri* appears to be related to acetic acid production. Yeast can be inhibited by the presence of short-chain fatty acids, like acetic acid, which penetrate by passive diffusion into cells and release hydrogen ions, thus decreasing intracellular pH quickly and resulting in cell death (Ruser and Kleinmans 2005).

The aim of this study was to determine the effects of wilting and *Lactobacillus buchneri* 40788 inoculation on fermentation end products, DM recovery, nutritive value and aerobic stability in triticale and fava bean intercrop silages grown organically under prevailing weather conditions in wet temperate coastal areas.

Material and methods

Forages and silages

This study was conducted on an experimental farm located in the North of Spain (latitude 43.23°N, longitude 6.07°W, altitude 65 m above sea level, Asturias, Spain). The average temperature for the growing period was 10 °C (ranging between 1 to 16 °C) with 78% relative humidity and 425 mm of rainfall.

A winter bi-crop of triticale and fava bean (x *Triticosecale* Wittm. and *Vicia faba* L.) was grown under organic conditions. This bi-crop was established in February 2005, on an old low-input mixed sward (*Lolium perenne* and *Trifolium repens*) previously used for grazing and without NPK inorganic fertilizers prior to and during this assay. Sowing was carried out on an experimental single plot area of 360 m². The viable seeding rate was 159 grains m⁻² for triticale and 26 seeds m⁻² for fava bean. After 14 weeks of growth, plants were harvested in May 2005 by direct cut method using a mower of cutting bars. The growth stage of forages at the time of harvest was milky grain stage for triticale and pods with grain for fava bean (Fraser et al. 2001). At that time, the triticale-fava bean ratio obtained was 6.3:1. The total yield obtained after harvest was divided in two parts: 1) triticale-fava bean after discarding the weeds from the existing sward (TF), and 2) fava bean alone (F).

Before ensiling, TF and F samples were divided in half, with one part being prepared for ensiling in direct cut (D) and the other wilted for 24 hours (W). All fractions to ensiling were cut at 2 cm using an ORGO precision chopper (Agro ORGA S.A.,

Tarragona Spain) and after that ensiled with addition (A) or no addition (NA) of *Lactobacillus buchneri* NCIMB strain 40788 (Lallemand Animal Nutrition, BP 59, Cedex, France). The additive was prepared diluting 200 g in 40 l of water, and applying one litre of this solution by ton of forage, to obtain a final application rate of 1×10^5 cfu g⁻¹ of fresh forage.

All material was ensiled at room temperature (20 ± 5 °C) in laboratory silos made of PVC cylinders provided with bun valves to allow for gas losses, and glass containers to store the evacuated effluent. These laboratory silos have a capacity of 4 dm³ and a forage density of 650 kg m⁻³, according to Martínez-Fernández and de la Roza-Delgado (1997). The amount of plant material ensiled was 2.5 ± 0.25 kg per silo, and three replicates per treatment. A total of 24 laboratory silages were made. At the end of the fermentative process (80 days), the silos were opened and sampled for analysis. Effluent production was measured throughout the process by weekly weighting.

Analytical methods

Forages

Two representative fresh forage samples for total herbage mass were taken and later each one was divided in two parts: TF and F (see forages and silages section). All samples (in direct cut and wilted) were analyzed in duplicate for ensilability characteristics, including water soluble carbohydrates (WSC) following Hoffman (1937) and buffer capacity (BC) using the methodology described by Playne and McDonald (1966). The fermentability coefficient (FC) was calculated according to Schmidt et al. (1971), cited by Weissbach (1999). Dry matter in forages (DM) was determined by drying in an air-forced oven at 102 °C for 24 h. For analytical determinations, a subsample from each treatment was dried at 60°C and milled at 0.75 mm. Samples were analyzed for ash and crude protein (CP) according to AOAC (1984), neutral detergent fiber (NDF) (Van Soest et al. 1991) and cellulase

digestibility (Riveros and Argamenteria 1987) to estimate metabolizable energy (ME) by ARC (1980).

Silages

After 80 days silos were opened and three sub-samples of each experimental silage were collected. The first sub-sample was pressed to obtain juice extract to determine pH, ammonia-N, lactic acid and volatile fatty acids. Ammonia-N was determined by UV-Vis (Ammonium test, Merck, Germany). Lactic acid and volatile fatty acids analyses were performed by HPLC with a Water Alliance 2690 instrument equipped with a Waters 996 Photodiode Array Detector Module (Waters, Milford, Massachusetts) monitoring at 206 nm and drove by Millennium software. The juice extract were analyzed on a Shodex RSpak KC-811 column (Waters), using a mobile phase with 0.025 % phosphoric acid. Flow rate was 1.0 ml min⁻¹ and column temperature for analyses was kept at 40° C.

The second silage sub-sample was freeze-dried to avoid loss of volatile compounds. After that, samples were milled at 0.75 mm and analyzed for DM, ash and CP (AOAC 1984), NDF (Van Soest et al. 1991) and cellulase digestibility to estimate metabolizable energy (ME) by ARC (1980).

The third silage sub-sample around 1.5±0.25 kg was used for aerobic stability analysis, defined by Moran et al. (1996) as the number of hours the silage mass remained at the baseline temperature before rising 2 °C. Following McEniry et al. (2007), this sub-sample was placed in a polystyrene box (60 × 40 × 15 cm) provided with no hermetic covers and exposed to the air in a room with a controlled temperature of 20±1 °C. Thermocouples were placed in the middle of the silage in each box and the temperature was automatically recorded each hour for 240 h. Silage pH was measured directly each day at different positions of the silages using a portable electrode (Inlab 427, Mettler Toledo).

To calculate losses, the material was weighted both in and out of the silos. The proportion of total DM losses was calculated according to the following expression:

$$Losses = 100 - \left(\frac{Silage(g) \times DM_{silage}}{Forage(g) \times DM_{forage}} \times 100 \right)$$

Statistical Analysis

Forages data were analyzed to ANOVA by the general linear procedure of SAS (SAS 1999). All silage data were analyzed as a factorial design with type of forage (S), wilting (W) and inoculation (A) as the main factors using again the SAS (1999) general linear models procedure. The model used was:

$$Y_{ijkl} = \mu + S_i + W_j + A_k + SW_{ij} + SA_{ik} + WA_{jk} + SWA_{ijk} + \epsilon_{ijkl}$$

where: Y_{ijkl} = observation, μ = population mean, S_i = forage effect ($i = 1$ to 2), W_j = wilting effect ($j = 1$ to 2), A_k = additive effect ($k = 1$ to 2), SW_{ij} = interaction between forage and wilting effects, SA_{ik} = interaction between forage and additive effects, WA_{jk} = interaction between wilting and additive effects, SWA_{ijk} = interaction between forage, wilting and additive effects and ϵ_{ijkl} = residual error. Significant differences were accepted if $p \leq 0.05$.

Aerobic stability was evaluated using an ANOVA with repeated measures testing for statistical significance of pH and temperature data, collected over 10 days and considering the type of forage, treatment by wilting and additive as fixed effects. The statistical analyses were performed using the SAS (1999) statistical package.

Results

The ensilability characteristics, chemical composition and estimated ME of fresh forages before ensiling are shown in Table 1. DM was below 200 g kg⁻¹ in fresh crops and was increased by wilting to about 320 g kg⁻¹. The bi-crop (TF) had higher DM (upper than 20%) and lower CP (around 50%) proportions than fava bean in direct cut and wilted forages, respectively ($p < 0.001$). After wilting, significant differences were observed with respect to chemical composition in both forages, with losses in CP and NDF ($p < 0.01$).

Table 1: Ensilability characteristics, chemical composition and energy content of forages before ensiling.

	Triticale-Fava bean		Fava bean		s.e.m	Significance		
	Direct	Wilting	Direct	Wilting		S	W	S×W
Ensilability characteristics								
Dry matter (g kg ⁻¹)	206	361	183	280	22.4	***	***	***
WSC (g kg ⁻¹ DM)	201	251	94	142	2.3	***	***	NS
BC (meq NaOH kg ⁻¹ DM)	218	127	319	221	40.0	***	***	NS
FC	28	52	21	33	1.9	***	***	***
Chemical composition and estimated energy								
Crude Protein (g kg ⁻¹ DM)	117	111	173	166	0.6	***	**	NS
NDF (g kg ⁻¹ DM)	550	534	519	491	2.1	***	**	NS
ME (MJ kg ⁻¹ DM)	9.5	9.5	9.3	8.9	0.03	***	*	*

Significant levels: *, ** and *** at p -values < 0.05, 0.01, 0.001, respectively. NS: $p \geq 0.05$. s.e.m.: standard error mean; W: Wilting effect; S: Forage effect; WSC: Water soluble carbohydrates; BC: Buffer capacity; FC: Fermentability coefficient; NDF: Neutral detergent fibre; ME: Metabolizable energy.

Fermentability coefficient was higher in TF than F for direct cut and wilting respectively ($p < 0.001$), as a consequence of the higher proportion of water soluble carbohydrates (WSC) and lower buffer capacity (BC) of the former. Furthermore, wilting for 24 hours improved the ensilability conditions in TF and F, increasing WSC and markedly reducing BC. The interaction between the effect of forage and wilting was also significant ($p < 0.001$).

Table 2 shows the chemical and fermentative characteristics on direct cut and wilted silages inoculated with or without *Lactobacillus buchneri* at ensiling.

The average DM losses were around 20 % for TF and F. In direct cut forages DM losses were higher in F than TF, it is inversely related with forage DM content. These losses decrease in wilted forages. As regards the additive effect, losses were higher in both TF and F silages with additive compared to those without inoculation ($p < 0.001$).

The total effluent production in direct cut without additive was higher in F than TF forages ($p < 0.05$), in concordance with their lower DM content (Table 2). In fact, twenty-four hours of wilting was enough to avoid effluent production during the fermentation period.

Wilting did not affect silage pH, although significant differences were observed in terms of the forage effect ($p < 0.01$). The pH was higher in F silages. Wilting time did not affect the chemical composition of silages, except for DM content ($p < 0.001$). The differences due to forage effect showed higher values of ash and CP and a lower proportion of DM and NDF in F than TF silages, respectively ($p < 0.001$), when comparing the direct cut and wilting effect. ME was not affected by the considered effects (S and W).

Attending fermentative characteristics, wilting promoted a substantial decrease in the proportion of NH₃-N ($p < 0.001$) without any effect on CP content, being ammonia synthesis lower in F than TF silages (S × W, $p < 0.05$). TF had a lower proportion of lactic and propionic acids and a higher proportion of acetic acid than F, the lactic/acetic acid ratio being lower in TF despite their better FC.

Addition of *Lactobacillus buchneri* at ensiling increased pH ($p < 0.001$). The inoculation also increased concentrations of ashes ($p < 0.001$), CP ($p < 0.01$) and NDF ($p < 0.01$), while decreasing metabolizable energy ($p < 0.05$).

As regards fermentative characteristics, the concentration of lactic acid was lower in silages

Table 2. Chemical and fermentative characteristics on direct cut and wilted silages inoculated with or without *Lactobacillus buchneri* at ensiling.

Parameter	Triticale-Fava bean				Fava bean				Significance							
	Direct		Wilting		Direct		Wilting		S	W	A	S×W	S×A	W×A	S×W×A	
	NA	A	NA	A	NA	A	NA	A								
pH	3.94	4.28	4.09	4.18	4.06	4.67	4.18	4.55	0.013	**	NS	***	NS	**	***	NS
Dry matter (g kg ⁻¹)	232	212	339	317	181	178	245	231	1.066	***	***	***	***	***	NS	NS
Ash (g kg ⁻¹ DM)	49.3	55.6	46.4	54.7	55.7	61.3	60.2	62.0	0.400	***	NS	***	*	NS	NS	NS
Crude protein (g kg ⁻¹ DM)	125	137	119	134	185	213	200	212	2.325	***	NS	**	NS	NS	NS	NS
NDF (g kg ⁻¹ DM)	554	594	552	601	494	490	463	512	5.894	***	NS	**	NS	NS	NS	NS
ME (MJ kg ⁻¹ DM)	9.11	8.67	8.97	8.59	9.41	9.26	9.33	8.25	0.080	NS	NS	*	NS	NS	NS	NS
Ammonia-N (g NH ₃ kg ⁻¹ N)	38.1	35.9	13.1	12.0	28.7	30.5	10.3	25.4	0.711	NS	***	*	NS	***	*	NS
DM losses (g kg ⁻¹)	163	218	145	222	206	258	165	215	4.680	***	NS	***	NS	NS	NS	NS
Effluent (l t ⁻¹)	80.5	52.9	0	0	96.6	43.2	0	0	3.391	NS	***	*	NS	NS	*	NS
Lactic acid (g kg ⁻¹ DM)	66.6	24.5	38.9	29.4	96.5	23.9	82.7	24.8	1.050	***	***	***	NS	***	NS	*
Acetic acid (g kg ⁻¹ DM)	48.5	88.9	39.5	66.6	37.3	60.6	25.8	64.6	1.069	***	***	***	NS	NS	**	**
Propionic acid (g kg ⁻¹ DM)	1.67	3.93	2.33	4.77	2.83	20.4	8.60	10.8	0.221	***	NS	***	NS	*	***	***
Butyric acid (g kg ⁻¹ DM)	6.53	3.57	5.03	4.30	7.20	7.17	6.20	0.00	0.651	NS	NS	NS	NS	NS	NS	NS

Significant levels: *, ** and *** at *p*-values < 0.05, 0.01, 0.001, respectively. NS: *p* ≥ 0.05. s.e.m.: standard error mean; S: Forage effect; W: Wilting effect; A: Additive effect
 NA: No additive; DM: Dry matter; NDF: Neutral detergent fibre; ME: Metabolizable energy.

treated with *L. buchneri* ($p < 0.001$). In contrast, silages inoculated resulted in a marked increase in the concentration of acetic ($p < 0.001$) and propionic ($p < 0.001$) acids. The additive only increased $\text{NH}_3\text{-N}$ synthesis in F, numerically but not statistically ($S \times A, p > 0.05$).

Additive treatment increased pH in both forages ($A, p < 0.001$) while the effect was more clear in F ($S \times A, p < 0.01$), pH was lower in TF than in F ($p < 0.01$). Moreover, the additive showed a strong influence on lactic ($p < 0.001$) and propionic ($p < 0.05$) acids levels in F.

Wilting appeared to inhibit the effects of *L. buchneri* on fermentation parameters because propionic acid (7.77 vs 12.2 g kg^{-1}DM) and $\text{NH}_3\text{-N}$ (18.7 vs 33.4 g $\text{NH}_3 \text{kg}^{-1}\text{N}$) were 40% less of those produced in direct cut inoculated silages ($W \times A, p < 0.001$). Besides acetic acid (65.6 vs 74.7 g kg^{-1}DM) decreased around 20% ($W \times A, p < 0.01$) without differences concerning lactic acid production (27.1 vs 24.2 g kg^{-1}DM).

Chemical parameters of silages were unaffected by interaction among forage, additive and wilting effects, whereas all fermentative characteristics except butyric acid concentration, were affected by this triple interaction. In this sense, in TF forages, with higher ensilability than F, the additive action was less effective reducing lactic acid ($S \times W \times A, p < 0.05$) and increasing acetic ($S \times W \times A, p < 0.01$) and propionic ($S \times W \times A, p < 0.001$) acids. In fava bean (F), with lower ensilability than TF, the additive effects were better than TF, especially in direct cut silages. In the other hand, the additive increases the ammonium concentration in silages with high proteolysis such as wilted fava bean silages ($S \times W \times A, p < 0.05$).

Regardless aerobic stability, all the silages involved in this study remained stable at least 10 days after opening. No heating above room temperature ($20 \pm 1^\circ\text{C}$) was observed during exposure to air (Table 3). Nevertheless, it should be stressed that according to the statistical analysis of repeated

Table 3. Changes in temperature during air exposure of silages depending on effects: forage, wilting and additive.

Day	Triticale-Fava bean				Fava bean			
	Direct		Wilting		Direct		Wilting	
	NA	A	NA	A	NA	A	NA	A
1	18.04±0.23	18.48±0.21	18.88±0.23	18.84±0.50	19.36±0.24	19.12±0.36	19.38±0.32	18.58±0.29
2	17.10±0.20	16.84±0.19	17.56±0.18	17.36±0.40	16.98±0.43	17.80±0.25	17.82±0.27	18.12±0.16
3	18.42±0.11	18.98±0.04	18.36±0.05	17.94±0.44	18.34±0.09	18.42±0.13	18.02±0.25	18.70±0.24
4	17.84±0.19	17.80±0.18	18.12±0.11	17.78±0.13	17.96±0.30	17.88±0.13	18.48±0.20	17.96±0.24
5	17.68±0.19	17.82±0.17	18.20±0.16	17.92±0.47	18.22±0.08	18.00±0.20	17.94±0.44	17.96±0.33
6	17.62±0.13	17.86±0.21	18.10±0.23	18.18±0.36	18.14±0.33	17.82±0.08	17.90±0.19	18.04±0.11
7	18.70±0.24	18.10±0.21	18.34±0.09	19.08±0.41	18.28±0.13	18.24±0.27	18.98±0.24	18.86±0.15
8	18.70±0.32	18.16±0.24	19.20±0.22	18.78±0.34	18.44±0.27	19.24±0.11	19.06±0.09	18.78±0.27
9	19.16±0.18	18.34±0.10	18.90±0.35	19.06±0.28	18.14±0.21	17.90±0.23	18.22±0.33	18.20±0.46
10	19.74±0.19	19.60±0.24	19.48±0.22	19.58±0.29	19.36±0.09	18.70±0.39	18.80±0.46	18.70±0.42

NA: No Additive; A: Additive.

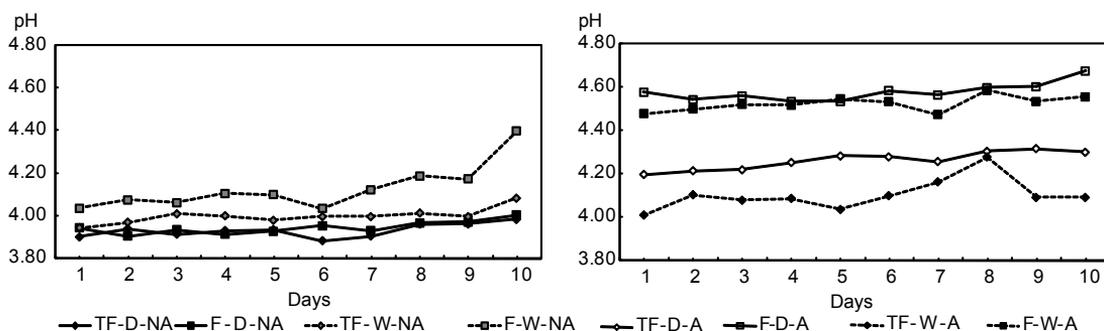


Figure 1. Changes in pH during aerobic deterioration of triticale-fava bean (TF) and fava bean (F) silages, ensiled directly (D) or wilted (W) and pre-treated with (A) or without (NA) *Lactobacillus buchneri*.

measures in time, no effects were found with respect to the additive. Marginal differences in final temperature were observed when comparing wilting and direct cut silages after exposure to air.

Figure 1 shows the evolution of pH over time. pH was significantly affected by the type of forage (4.12 vs 4.41 for TF and F, respectively; $p < 0001$) and inoculation (4.41 vs. 4.07 for silage with and without additive, respectively; $p < 0001$). Furthermore, the forage-additive interaction ($p < 0.001$) with the lowest pH values corresponded to TF forage without additive (pH = 3.98) and the highest to F silages with additive (pH = 4.66). The $W \times A$ interaction ($p < 0.001$) shows that wilting depresses the *L. buchneri* effect related to pH evolution after silages aerobic exposure.

Discussion

The current study provides evidence that adding *L. buchneri* to direct-cut forages of triticale-fava bean and fava bean alone at ensiling increases DM losses. However, this effect decreases in wilted forages, probably because the higher dry matter content in wilted forages decreases the overall activity of *L. buchneri* and therefore less acetic acid is produced. This finding may be explained by the fact that the

addition of *L. buchneri* could have inhibited the activity of yeasts that were probably accelerated by wilting (Nishino and Touno 2005, Oude Elfernick et al. 2001).

The DM content in wilted silages was clearly lower after ensiling than before it. Similar effects were found by Nishino and Touno (2005) on wilted grass silages, and it was explained by gas losses during fermentation process. Interaction in DM content could be explained due to the fact that the proportional increase in DM in F was higher than in TF. When F was wilted before ensiling, the ash content decreased due to leaf loss, while this effect was the opposite in TF. The results of this study showed that wilting forage for 24 hours prior to ensiling reduced lactic and acetic fermentation regardless of the type of forage.

Nishino and Touno (2005) indicated that DM loss was significantly increased when *L. buchneri* was inoculated into direct-cut materials, in our study this effect was numerically but not statistically significance.

As expected, inoculation with *L. buchneri* at ensiling altered the fermentation parameters by causing an accumulation of acetic and propionic acids via the metabolism of heterofermentative *Lactobacillus* culture. These strains transform water soluble carbohydrates into lactic acid at an early stage of fermentation. This lactic acid is further transformed into acetic and propionic acids, the

combined action of which has antifungal properties. However, the result of this metabolic process is an increase in final pH. In this experiment, the pH of silages inoculated with *L. buchneri* was significantly higher than that of silages which were not inoculated. Moreover, the synthesis of acetic and propionic acids rose during the fermentation process with decreasing lactic acid content. Similar results have been reported with cereal silage by Kung and Ranjit (2001) using different rate of inoculants; alfalfa silage by Kung et al. (2003) after 56 days of ensiling in laboratory silages; whole plant corn silage (Kleinschmit and Kung 2006) or different crop silages (Ruser and Kleinmans 2005). Nishino and Touno (2005) have also reported that in direct-cut grass silages pH was lower than 4.0 and inoculants treated grass silages had pH values around 4.5. In contrast, Wróbel (2008) also added commercial inoculants containing homo and heterofermentative lactic acid bacteria to grass forages but found no increases in the pH values of the resulting silage.

When forages were inoculated at ensiling with *L. buchneri*, the final silages contained lower concentrations of lactic acid than untreated forages. Conversely, a marked increase in the concentrations of acetic and propionic acid were observed. In a previous study, Combs and Hoffman (2001) reported that silages inoculated with an effective dose (4 to 6×10^5 cfu g⁻¹ of fresh material) of *L. buchneri* had higher concentration of acetic acid and lower levels of lactic acid than untreated silages. Similar effects were found by Kung et al. (2003) without differences among doses of inoculants.

The ammonia-N concentration of fava bean silages were increased by inoculation treatment in agreement with Kung et al. (2003) working with alfalfa silages.

In the current study, all silages had very high acetic acid concentrations, which have contributed to the high aerobic stability of them. Although the amount of acetic acid obtained as a result of the W × A interaction (65.6 g kg⁻¹ DM) did not reach the values obtained in direct cut silages with additive (74.7 g kg⁻¹ DM), it did remain significantly higher than in direct cut silages without additive (42.9 g kg⁻¹ DM). Combs and Hoffman (2001) related the beneficial impact of *L. buchneri* to the production

of acetic acid. In fact, aerobic stability could be improved because acetic acid inhibits growth of specific species of yeast that are responsible for heating upon exposure to air. Similar results have also been reported by Taylor et al. (2002) and with those obtained by Nishino and Touno (2005) with Italian ryegrass and Festilolium.

Critics of using heterolactic acid bacteria as silage inoculants suggest that high concentrations of acetic acid in silages have had depressing effects on dry matter intake (DMI) for lactating cows, but at this time it is not clear whether enough acetic acid will be produced in silages treated with *L. buchneri* to affect feed intake (Combs and Hoffman 2001). Recently, Wróbel (2008) reported that the additive treatment using bacterial inoculants containing homo and heterofermentative lactic acid bacteria, in pre-wilted grass silages (about 450 g kg⁻¹ DM), and with very low concentrations of acetic acid (ranged between 10.1–14.5 g kg⁻¹ DM), did not affect silage intake and daily live weight gain of heifers.

Inoculation with *L. buchneri* in forages to make laboratory silages did not affect aerobic stability after 10 days of air exposure. The chosen temperature for the experiment ($20 \pm 1^\circ\text{C}$) may possibly be too low. In fact, during this study period, no heating above room temperature was observed in the silages. These results are in agreement with those obtained by Taylor et al. (2002), who reported that the temperature of barley silages from laboratory silos did not rise after exposure to air for seven days, and by Combs and Hoffman (2001), who explained that *L. buchneri* is unlikely to improve silage quality in situations where silage has a history of being aerobically stable at feed out. Our location was probably optimum to maintain silage under stable conditions after exposure to air.

Conclusions

The results of this study show that the association of triticale with fava bean for ensiling improves ensilability characteristics when compared with

fava bean alone. In fact, wilting forage for twenty-four hours before ensiling also increases the fermentability coefficient. Furthermore, this wilting period is sufficient to avoid effluent losses during the fermentation process and to reduce ammonia nitrogen production without changes in CP content.

Lactobacillus buchneri promotes changes in the amount of fermentation end products, leading to acetate and propionate from lactic acid via the metabolism of heterofermentative biological cultures.

Wilting appeared to depress, but not inhibit the effects of *Lactobacillus buchneri* on the fermentation parameters. The inhibitory effects of *Lactobacillus buchneri* on aerobic stability were not confirmed in this study.

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